EXHIBIT 5

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Immunology

cDNA sequences of two inducible T-cell genes

(unidentified T-cell mediators/cDNA/concanavalin A-inducible)

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ABSTRACT **We have previously described a set of human T-lymphocyte-specific cDNA clones isolated by a modified differential screening procedure. Apparent full-length cDNAs containing the sequences of 14 of the 16 initial isolates were sequenced and were found to represent five different species of mRNA; three of the five species were identical to previously reported cDNA sequences of preproenkephalin, T-cell-replacing factor, and a serine esterase, respectively. The other two species, 4-1BB and L2G25B, were inducible sequences found in mRNA from both a cytolytic T-lymphocyte and a helper T-lymphocyte clone and were not previously described in T-cell mRNA; these mRNA sequences encode peptides of 256 and 92 amino acids, respectively. Both peptides contain putative leader sequences. The protein encoded by 4-1BB also has a potential membrane anchor segment and other features also seen in known receptor proteins.**

Most T-cell factors have been classically identified by recognizing biologic activities in assays, purifying the protein, and then isolating the corresponding genes using bioactivities and protein information. An alternative approach is to isolate putative T-cell genes based upon specific expression and then to demonstrate the function of the unknown molecule (1–3). This laboratory has recently cloned a series of T-cell subset-specific cDNAs from cloned helper T-lymphocyte (HTL) L2 cells and cloned cytolytic T-lymphocyte (CTL) L3 cells by employing a modified differential screening procedure (4). Subsequently, we isolated and sequenced apparent full-length cDNAs corresponding to 14 of the original clones representing five different species of mRNA. Here we summarize our studies and present the sequences of two cDNAs that do not correspond to previously reported T-cell mRNA species.§

MATERIALS AND METHODS

Cells. We are grateful to F. Fitch of the University of Chicago for supplying us with the T-cell clones, L2 and L3 cells (5). Cloned murine CTL L3 cells are Thy-1,2$^+$, Lyt-2$^+$, LFA-1$^+$, L3T4$^-$, and H-2L^d-reactive. Cloned murine HTL L2 cells are Thy-1,2$^+$, LFA-1$^+$, Lyt-2$^-$, L3T4$^+$, and Mls$^{a/d}$-reactive.

Before the isolation of RNA L2 cells were stimulated with Con A (10 μg/ml) for 14 hr at a cell concentration of 10^6–10^7 cells per ml. L3 cells were stimulated with Con A (2 μg/ml) for 14 hr at a cell concentration of 2.5×10^6 cells per ml. Mouse thymoma EL-4 cells (6) were stimulated with phorbol 12-myristate 13-acetate (10 ng/ml) at a cell concentration of 1.0×10^6 cells per ml for 20 hr; stimulation was monitored by interleukin-2 assay (7). RNA was isolated from the unstimulated B-cell lymphoma K46 (8) and rat natural killer cell large granular lymphocytes (9).

Isolation of T-Cell-Specific cDNA Clones. We have previously isolated a group of cDNAs that are specific for T cells in contrast with B cells, employing both positive and negative differential screening and RNA blot analysis of various lymphoid cells. The T-cell-specific cDNAs were further studied to determine whether they were specific for cloned HTL L2 cells or cloned CTL L3 cells. This allowed us to isolate T-cell-subset transcripts that exist at a low level (4). The two cDNA sequences, designated as L2G25B and 4-1BB, were isolated in this fashion and were referred to as L2G25#4 and L3G29#4, respectively, in an earlier report (4).

RNA and DNA Blot Hybridization. Total cytoplasmic RNA or poly(A)$^+$ RNA was fractionated on 1.2% agarose-formaldehyde gels and transferred to GeneScreen*Plus* (NEN). Gel-purified cDNA inserts were ^{32}P-labeled by nick-translation and used as probes. When a Northern (RNA) blot of GeneScreen*Plus* was used multiple times for hybridization, the previous probe was removed by placing the membrane in 10 mM Tris·HCl (pH 7.0)/0.2% sodium dodecyl sulfate (SDS) at 85°C for 1 hr.

High-molecular weight DNA of mouse spleens was prepared as described (10). Restriction endonuclease digests of DNA were electrophoresed in 0.8% agarose gel at 4°C. The DNA was denatured and transferred to GeneScreen*Plus* as described by Southern (11). The blot was hybridized with ^{32}P-labeled cDNA.

Screening of cDNA Library and DNA Sequencing. L2 and L3 cDNA libraries that had previously been prepared (4) were rescreened with cDNA inserts of each of the 14 T-cell-specific cDNA fragments. Typically 10 positive clones were chosen for each species, and the sizes of cDNA inserts were determined. The longest cDNA inserts were employed for nucleotide sequencing by the dideoxynucleotide chain-termination technique (12) employing Sequenase (United States Biochemical), with modifications made to accommodate 2′-deoxyadenosine 5′[α-^{35}S]thiotriphosphate (13).

Nucleotide and Protein Sequence Comparison. Full-length cDNA and predicted protein sequences were compared with the sequences in the National Institutes of Health GenBank Genetic Sequence Databank (Release 56), European Molecular Biology Laboratories (Release 16.0), and National Biomedical Research Foundation (Release 17.0). Predicted proteins were analyzed by the PEPPLOT program.

RESULTS

Table 1 summarizes T-cell cDNA identified from 14-hr Con A-stimulated L2 and L3 cDNA libraries. Besides the cDNAs listed in the table, granulocyte/macrophage colony stimulating factor, interleukin 2, interleukin 3, T-cell antigen receptor α- and β-chain, and c-*myc* cDNAs were identified by cross-

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Abbreviations: CTL, cytolytic T lymphocyte; HTL, helper T lymphocyte.

§The sequences reported in this paper are being deposited in the EMBL/GenBank data base (accession nos. J04491 and J04492).

Table 1. Summary of cDNA clones identified

Full-length cDNA	cDNA clone isolated previously*	Specificity of expression	Identification
4-1BB	L3G29#4	L2 and L3	Unknown
	L3G25#4	L2 and L3	
	L3G14#2		
L2G25B	L2G25#4	L2 and L3	Unknown (related to cDNA PLD78)
	L2G95#4		
	L2G53#3		
	L2G95#3		
L2S35	L2S35#3	L2 only	Preproenkephalin
	L2PBK671		
8-1R	L2PBK791	L2 and EL-4	T-cell-replacing factor
	L2PBK642		
	L2PBK631		
L3G10	L3G10#6	L3 only	*HF* gene (serine esterase)
	L3G18#3		
ND†	L3G7#1	L3 and EL-4	Unknown
ND†	L3G26#1	L3 and EL-4	Unknown

*cDNA clones were isolated independently and described as separate clones in the previous report (4).
†Full-length version of the two clones was not isolated.

hybridization of T-cell-enriched cDNAs with the corresponding full-length cDNA provided by other laboratories.

Among the 16 unidentified T-cell cDNA clones isolated by this screen, two clones represented preproenkephalin that was identical to the sequence reported by Zurawski *et al.* (14), three clones were T-cell-replacing factor (15), and two clones represented a T-cell serine esterase gene (16).

In the previous studies (4) we had also isolated an additional 13 L3 cDNAs whose specificity could not be assigned by RNA blot analysis because of the weak signals. One of them (L3-1) was 64% homologous to a T-cell serine esterase (17). The sequence has been reported as a new member of the T-cell serine esterase family (18). The other 12 clones have not yet been analyzed.

Four species were from different regions of a cDNA referred to as L2G25B (800 base pairs). L2G25B was homologous to a human cDNA PLD78 (19) of unknown function. Three species (L3G29#4, L3G25#4, and L3G14#2) were from different regions of 4-1BB (2400 base pairs). We have found no reports of sequences homologous to 4-1BB. L3G7#1 and L3G26#1 were not characterized vigorously because they were represented at low levels in T-cell mRNA and were not inducible.

Fig. 1*A* shows the nucleotide and deduced amino acid sequence of the longest open reading frame of L2G25B. The open reading frame codes for 92 amino acids including a putative signal sequence. The deduced sequence of the first 23 amino acid residues has characteristics of the signal peptide of secretory proteins (23, 39). Therefore the mature protein is composed of 69 amino acids with a M_r of 7880. No potential N-glycosylation sites or transmembrane-like domain exists. The 3′-untranslated region has repeated occurrences of the sequence AUUUA (40) characteristic of lymphokines and other rapidly degraded mRNA.

An optimum alignment between L2G25B and PLD78 is shown in Fig. 1*B*. The identity of amino acids between the two proteins was ≈80%. The evolutionary conservation of these molecules from mouse to human may indicate that they play an important role in T-cell function. Recently a family of lymphokine genes termed RANTES (regulated upon *a*ctivation, *n*ormal *T* expressed, and presumably *s*ecreted) has been defined to include PLD78 (20). After the present manuscript was prepared, a report appeared describing a macrophage inflammatory protein (21); the predicted amino acid sequence of this monokine is identical to that of L2G25B.

The nucleotide sequence of three overlapping cDNA clones represented by 4-1BB was determined (Fig. 2). The nucleotide sequence of 4-1BB revealed a single long open reading frame, beginning with the ATG codon at nucleotide

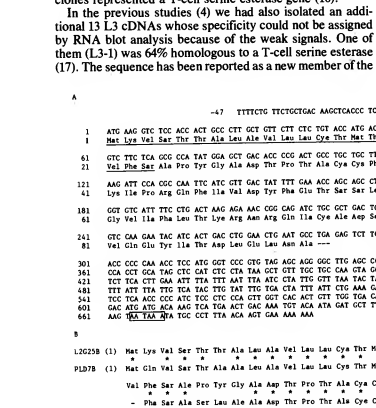

FIG. 1. (*A*) Nucleotide sequence of L2G25B and the deduced amino acid sequence. Nucleotide sequence of the message strand is numbered in the 5′ and 3′ direction. The 5′ noncoding sequence is indicated by negative numbers. Nucleotide residue 1 is the first nucleotide of the ATG initiation codon. The predicted amino acid sequence is shown below. Potential signal peptide is underlined, consensus polyadenylylation signal is boxed, and stop codon is indicated by ---. (*B*) Optimum alignment between L2G25B and PLD78-deduced amino acids. Homology search revealed that the amino acid sequence of L2G25B showed an extensive homology with a reported human sequence, PLD78 (15) of unknown function, whose expression is inducible by phorbol 12-myristate 13-acetate in human tonsillar lymphocytes. The identity of amino acids between the two proteins was ≈80%. *, Identical amino acids in these proteins; +, chemically similar amino acids found in both sequences; Δ, a potential cleavage site of the signal peptide.


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 -145
 -140    CATGAACTCC TGAGTGGATA AACAGCACCC GATATCTCTC TCTAAACCAA TATTACTACA CCAGGAAAAG
  -70    GACACATTCG ACAACACGAA AGGAGCCTGT CACACAAAAC CACAGTCTCC TCTCCATCTG ACATTTCGCC
    1    ATC CCA AAC AAC TCT TAC AAC CTG CTC GTC ATT CTG CTC CTC CTA CTC GGC TCT CAC AAC   60
    1    Met Cly Asn Asn Cys Tyr Asn Val Val Val Ile Val Leu Leu Leu Val Gly Cys Clu Lys   20

   61    CTC CCA CCC CTC CAC AAC TCC TCT CAT AAC TCT CAC CCT GCT ACT TTC TCC ACA AAA TAC  120
   21    Val Cly Ala Val Cln Asn Ser Cys Asp Asn Cys Cln Pro Cly Thr Phe Cys Arg Lys Tyr   40
                                      •           •                       •

  121    AAT CCA CTC TCC AAC AGC TCC CCT CCA AGT ACC TTC TCC AGC ATA GGT GGA CAG CCG AAC  180
   41    Asn Pro Val Cys Lys Ser Cys Pro Pro Ser Thr Phe Ser Ser Ile Gly Cly Cln Pro Asn   60
                      •           •

  181    TGT AAC ATC TGC ACA CTG TCT CCA CCC TAT TTC AGG TTC AAG AAG TTT TCC TCC TCT ACC  240
   61    Cys Asn Ile Cys Arg Val Cys Ala Gly Tyr Phe Arg Phe Lys Lys Phe Cys Ser Ser Thr   80
          •           •           •                                       •

  241    CAC AAC CCG GAG TGT GAC TCC ATT GAA GCA TTC CAT TCC TTC CGG CCA CAC TCC ACC AGA  300
   81    His Asn Ala Glu Cys Glu Cys Ile Clu Cly Phe His Cys Leu Cly Pro Cln Cys Thr Arg  100
                          •       •                       •                   •

  301    TCT CAA AAG GAC TGC AGG CCT GGC CAC CAC CTA ACC AAC CAC CCT TCC AAA ACC TCT ACC  360
  101    Cys Glu Lys Asp Cys Arg Pro Cly Gln Clu Leu Thr Lys Cln Cly Cys Lys Thr Cys Ser  120
          •               •                                           •           •

  361    TTC CGA ACA TTT AAT CAC CAC AAC CCT ACT CCC CTC TCT CCA CCC TGG ACG AAC TGC TCT  420
  121    Leu Cly Thr Phe Asn Asp Cln Asn Cly Thr Cly Val Cys Arg Pro Trp Thr Asn Cys Ser  140
                                                          •                       •

  421    CTA CAC CCA ACC TCT GTC CTT AAC ACC GGG ACC ACG GAC AAG GAC CTC CTC TCT CCA CCC  480
  141    Leu Asp Cly Arg Ser Val Leu Lys Thr Cly Thr Thr Clu Lys Asp Val Val Cys Gly Pro  160
                                                                              •

  481    CCT CTC CTC ACC TTC TCT CCC AGT ACC ACC ATT TCT CTC ACT CCA GAG GGA GGA CCA CCA  540
  161    Pro Val Val Ser Phe Ser Pro Ser Thr Thr Ile Ser Val Thr Pro Clu Cly Cly Pro Cly  180

  541    CGG CAC TCC TTG CAC CTC CTT ACC TTC TTC CTG GCG CTG ACA TCC CCT TTG CTG CTG GCC  600
  181    Cly His Ser Leu Cln Val Leu Thr Leu Phe Leu Ala Leu Thr Ser Ala Leu Leu Leu Ala  200

  601    CTC ATC TTC ATT ACT CTC CTC TTC TCT CTC CTC AAA TGG ATC AGG AAA AAA TTC CCC CAC  660
  201    Leu Ile Phe Ile Thr Leu Leu Phe Ser Val Leu Lys Trp Ile Arg Lys Lys Phe Pro His  220

  661    ATA TTC AAC CAA CCA TTT AAC AAC ACC ACT CGA GCA GCT CAA CAC CAA GAT GCT TCT ACC  720
  221    Ile Phe Lys Cln Pro Phe Lys Lys Thr Thr Cly Ala Ala Gln Clu Clu Asp Ala Cys Ser  240
                                                                                  •
  721    TCC CCA TCT CCA CAG GAA GAA CAA CCA CCA GGA GGA GGC TAT CAC CTG TGA TGTACTATC    780
  241    Cys Arg Cys Pro Cln Glu Glu Glu Gly Cly Cly Cly Gly Tyr Clu Leu ---
          •       •

  781    CTAGGACATC TCTGGCCCCA AACCCAGAAG CACTAGGACC CCACCATCCT CTGGAACACC ACAAGCAACC   850
  851    CCACCACCCT GTTCTTACAC ATCATCCTAG ATGATGTGTG GGCGCGCACC TCATCCAAGT CTCTTCTAAC   920
  921    GCTAACATAT TTCTCTTTAC CTTTTTTAAA TCTTTTTTTA AATTTAAATT TTATCTGTGT CAGTGTTTTG   990
  991    CCTGCCTGTA TCCACACGTC TCTCTCTCTC TCTCTCTCAC ACTCCTCATC CCTGACCAGG TCAGAAGAGA  1060
 1061    AACGGTTGCT TCCATAACAA CTGGAGTTAT GGATGGCTGT GAGCCGGnnn GATAGGTCCC CACGGAGACC  1130
 1131    TGTCTTCTTA TTTTAACGTG ACTGTATAAT AAAAAAAAAA TGATATTTCG CGAATTGTAG ACATTCTCCT  1200
 1201    GACACCCTTC TAGTTAATGA TCTAACAGGA ATTCTTGATA CCTACTATAC TCTATATGTG TATCTATATC  1270
 1271    TATATCTATA TATAAGACTC TTTTACTCTC AAACTCAACC TACACTCTCT CCTTACCAGG TCAATTTTAT  1340
 1341    TGGACATTTT ACGTCACACA CACACACACA CACACACACA CACCTTTATA CTACCTACTCT TATCGGTAT  1410
 1411    TCTACGTCAT ATAATGGGAT AGGCTAAAAC CAAACCAAAG ACTCACTCAT ATTATTGTCCA GGTGACAGA  1480
 1481    CTACCCCTTC TGGGTACGTA GGGACACACC TCCTTCGGAC TCTCTAAAAC TCCCCTTAGA ACTCTCGTCA  1550
 1551    ACTTCCCCGA CGAAGAGGAC ACACGAGACA CAGTCCGAAA AGTTATTTTT CCGGCAAATC CTTTCCCTCT  1620
 1621    TTCCTGACAC TCCACCCCTT CTGGACACTT CAGTGTCATC CTTGCCCCCC AACCTCAGGT GGTACCCCTC  1690
 1691    TGTAGGGGCG GGGAGACACA GCCGCGGGGG AGCTACGAGA ATCGACTCAC ACCCCCCCCC CGGCTTCCCA  1760
 1761    AATGAAACTT TTTTAATCTC ACAAGTTTCC TCCGGGCTCC GCGGACCTAT GGCGTCGATC CTTATTACCT  1830
 1831    TATCCTGGCC CCAAGATAAA ACAACCAAAA CCCTTCACTC CGGTACTAAT TCTCCCTGCC GGCCCCCGTA  1900
 1901    AGCATAACGC GGCCATCTCC ACTTTAAGAA CCTCGCCCCG TTCTGCCTGG TCTCGCTTTC GTAAACGGTT  1970
 1971    CTTACAAAAG TAATTAGTTC TTGCTTTCAG CCTCCAAGGT TCTCCTACTC TATGGCACCA TCAACGGTGG  2040
 2041    TATTTGCTAC GGCTCACCGC TACGCCGCCG CAATAACCCT ACTCGGCGGC CCGTCGAAGG CCCTTTGGTT  2110
 2111    TCAGAAACCC AAGGCCCCCC TCATACCAAC CTTTCCACTT TCATTCTTCC CCCTACCTGG TCCTGGGTCC  2180
 2181    CTTAGCTCTT TCTCGATAGT TAC AC
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FIG. 2. Nucleotide sequence and the deduced amino acid sequence of 4-1BB. The nucleotides of the message strand are numbered in the 5′ and 3′ direction, and numbers are shown on both sides of the sequence. Nucleotide residue 1 is the adenine of the initiation codon ATG, and the nucleotides on the 5′ side of residue 1 are indicated by negative numbers. The predicted amino acid sequence is shown below the nucleotide sequence. Putative signal peptide and the potential asparagine-linked glycosylation sites are underlined; the potential transmembrane domain is double underlined. The potential polyadenylylation signal is boxed. N, ambiguous nucleotide sequences in the 3′ untranslated region; ---, stop codon; •, cysteine residues.

residues 1–3. This reading frame codes for a polypeptide of 256 amino acids with a M_r of 27,587. The assigned ATG is preceded by an in-frame termination codon TGA (nucleotide residues −12 to −9). The sequence flanking the assigned ATG (nucleotide residues −5 to 4) contains eight of nine residues identical to the consensus sequence (CCRCCATGG, where R represents guanosine or adenine) described by Kozak (22). The codon specifying the carboxyl-terminal leucine is followed by the translational termination codon TGA (nucleotide residues 769–771). Clone 4-1BB contains an unusually long 3′-untranslated sequence, which did not extend as far as the poly(A)$^+$ tail. A potential polyadenylylation signal is present at nucleotides 1158–1163 (Fig. 2, boxed). This signal may sometimes be functional because this gene produces at least two different sizes of mRNA. Fig. 3 *a* and *b* shows RNA blot analysis of Con A-stimulated L3 RNA. When the blot was hybridized to an L3G25#4 probe that contained sequences to the 3′ side of the polyadenylylation signal (nucleotides 1284–1557), the probe detected one RNA species of ≈2.4 kilobases (kb). When the same blot was hybridized to an L3G14#2 probe that contained sequences to the 5′ side of the polyadenylylation signal (nucleotides 661–855) the probe detected two mRNA species of ≈1.5 kb and 2.4 kb. Only one copy of the sequence ATTTA that is often present in multiple copies in the 3′-untranslated regions of unstable mRNA was present in 4-1BB, and further studies of the stability of its mRNA are needed. Northern blot analysis has shown that 4-1BB mRNA is inducible in both L2 and L3 cells and is present in several CTL clones and hybridomas but is absent in the EL-4 T-cell line (B.S.K., unpublished results).

The deduced sequence of the first 23 amino acids of 4-1BB cDNA has characteristics of the signal peptide of secretory and membrane-associated proteins (23) and fits the −1, −3 rule (24). We putatively assigned the first 23 amino acids as a signal peptide, although the presence of lysine at −4 and glutamic acid at −5 are somewhat unusual. Thus, the protein backbone of processed 4-1BB protein would be composed of 233 amino acids with a M_r of 25,167. Two potential asparagine-linked glycosylation signals (25, 26) are located at

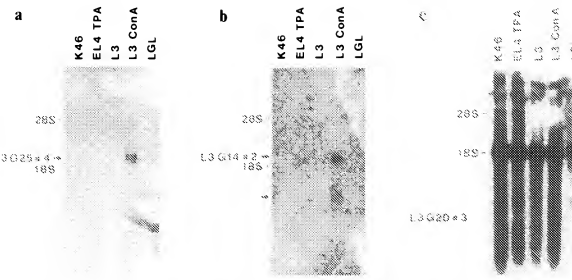

FIG. 3. Expression of two different sizes of 4-1BB mRNA. Ten micrograms of poly(A)$^+$ mRNA from mouse B-cell line (K46), phorbol 12-myristate 13-acetate-stimulated EL-4 (EL-4 TPA), and rat natural killer cell line (LGL) and 10 μg of total RNA from unstimulated L3 (L3) and Con A-stimulated L3 (L3 ConA) were fractionated on a 1.4% formaldehyde-agarose gel, transferred to GeneScreen*Plus*, and hybridized to ^{32}P-labeled L3G25#4 (*a*), L3G14#2 (*b*), and L3G20#3 (*c*) sequentially. L3G25#4 and L3G14#2 represent cDNA fragments of the 3′ side and 5′ side to boxed AATAAA sequence, respectively. L3G20#3 is an anonymous cDNA from L3 cDNA library and is used to show that each lane of the blot contains a similar amount of RNA. Positions of 28S and 18S rRNA markers are each indicated; arrows indicate the specific hybridization signals.

amino acid positions 128 and 138 as underlined in Fig. 2. The predicted 4-1BB protein contained an unusually large number of cysteines. There are 23 cysteine residues in the putative mature protein as marked with dots in Fig. 2. These residues are arranged with a spacing reminiscent of that seen in several groups of proteins including zinc finger DNA-binding proteins (27), epidermal growth factor receptor (28), the *Drosophila* Notch locus product (29), and certain translation factors (30, 31). Amino acids flanking certain of the cysteine groups also resemble sequences found in other proteins. For example, with a replacement of leucine by isoleucine, there is an exact match to seven of eight amino acids of a putative zinc finger structure in the yeast eIF-2β protein (31). These residues may represent metal binding sites, but, as discussed by others (32), their role in the functions of a protein cannot be uniquely predicted.

Following the cysteine-rich regions of the protein is a stretch of amino acids (residues 140–185) in which almost 30% of the amino acids are serine or threonine; these are potential sites for O-linked glycosylation and are reminiscent of those seen, for example, in the low density lipoprotein receptor (33). There is a stretch of 26 amino acids that constitutes a hydrophobic domain toward the carboxyl terminus of the protein (amino acids 186–211) (Fig. 4), followed by a sequence containing several basic residues. This region may serve as a membrane-spanning anchor domain.

Finally, the relatively short carboxyl-terminal putative cytoplasmic domain contains both two short runs of three and four acidic residues, respectively, and a sequence of five glycines followed by a tyrosine. The short stretches of acidic residues are also found in the cytoplasmic domain of the low density lipoprotein and atrial natriuretic peptide receptors, for example (33, 35).

Southern Blot Analysis. As shown in Fig. 5, a fragment of L2G25B and of 4-1BB cDNA each detects a single-restriction fragment of ≈15 kb and 18 kb in C57BL/6 and BALB/c DNA, respectively. The data indicated that the genes encoding the two molecules exist as a single copy in C57BL/6 and BALB/c mice.

DISCUSSION

We and others have developed protocols for differential screening of cDNA libraries by which one can detect a broad representation of the mRNA differentially expressed in two cell types (1–4). The method was applied to the systematic analysis of HTL and CTL gene expression and offered an alternative approach to classical protein purification for identifying effector molecules and genes. The genes for the T-cell antigen receptor, certain T-cell-specific effectors, and the probable gene mutated in an X chromosome-linked immunodeficiency syndrome of mice (36) were cloned and characterized in this fashion, and the set of clones described in this and a previous report were obtained before most of the individual species represented had been isolated by other methods.

A number of lymphokine clones, including interleukins 2, 3, and 5 and granulocyte/macrophage colony-stimulating factor as well as other T-cell-specific clones such as the serine esterases and T-cell receptors, were selected in the relatively small libraries (total of 18,000 L2 clones and 10,000 L3 clones) whose screening we have reported here and elsewhere. Probably the bulk of the most abundant T-cell-specific

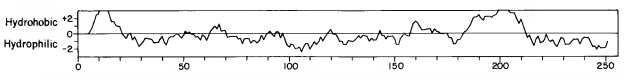

FIG. 4. Hydropathicity profile of the predicted amino acid sequence of 4-1BB. Local hydropathicity values calculated by the method of Kyte and Doolittle (34) were plotted versus amino acid residues. Positive values indicate hydrophobic regions, and negative values indicate hydrophilic regions.

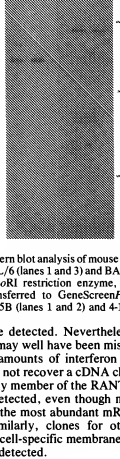

FIG. 5. Southern blot analysis of mouse genomic DNA. Genomic DNA from C57BL/6 (lanes 1 and 3) and BALB/c (lanes 2 and 4) was digested with *Eco*RI restriction enzyme, fractionated on a 0.8% agarose gel, transferred to GeneScreen*Plus*, and hybridized to ^{32}P-labeled L2G25B (lanes 1 and 2) and 4-1BB (lanes 3 and 4).

sequences were detected. Nevertheless, other potent biologic effectors may well have been missed. For example, L3 secretes large amounts of interferon γ, but, for unknown reasons, we did not recover a cDNA clone for this molecule. No clone for any member of the RANTES family other than L2G25B was detected, even though mRNA for such genes may be among the most abundant mRNA species produced in T cells. Similarly, clones for other T-cell activation antigens and T-cell-specific membrane channel-forming proteins were not detected.

Clones of the cDNA of 4-1BB were obtained from both the L2 and L3 cells. The clones corresponding to L2G25B were all isolated from L2 cells, and the possibility remains that the mRNA sequences detected in L3 corresponded to a different member of this gene family. The two species of cDNA reported here represent the two abundant, inducible sequences detected in both the HTL and CTL clones used in the present study. The induction properties of both clones resembled those of other lymphokines in that induction could be detected within 30 min after stimulation; they could both be induced either by Con A or by antigen stimulation. Unlike interleukin 2 receptor mRNA (37), induction of both mRNA species was blocked by treatment with cyclosporine A (B.S.K., unpublished work). Because 4-1BB protein was inducible in both T-cell clones, it may play roles in the T-cell activation process and function of the activated T cells less specific to a certain T-cell subset than those of the well-described lymphokines. As discussed above, 4-1BB has a number of properties reminiscent of membrane receptors and transmembrane signaling proteins. However, in at least the instances of the *Drosophila* Notch and sevenless loci (29, 38), an apparent transmembrane protein seems capable of acting directly or indirectly *in trans* on other cells.

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